The-over Heparin 40mL column -

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SD in $(NH_4)_2SO_4$ sol'n - @ 18,000 x g 40 minutes -
in GSA rota -
Save supernatant -
Save pellets -
Store one pellet in -20°C - process the other
pellet 2

pellet 1 slightly greater than half - ~3/5
pellet 2 slightly less than half - ~2/5

Resuspend pellet in 20 mL of Buffer 1 -

Buffer 1

5mM NMS pH 7.5
3% glycerol
20 mM KCl
5mM BME
.1mM PMSF

dialyze - against Buffer 1 for ~8hrs -
Exchange buffer 4 times -

Heparin column - use prepacked Heparin from A.G -
~40 mL column - pump w/ Buffer 1 + KCl -
wash w/ H_2O -

previously A.G used 3mL Heparin a
5 gram crack

Direct scale up = 3/5 = 4/~50gr = 30mL Heparin

equilibrate w/ Buffer 1 → (Note: made 20mM KCl)

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sed & Understood by me,	Date	Invented by S. Hynes	Date
Mary Forso	4/5/95	Recorded by	03/30/95

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Conductivity of Load - 28 mS - after ~8 hrs of dialysis

Notice a small precipitate matter in dialysis tube - spin down in SS-34 - 18,000 x g - 10 minutes - save pellet - small + white -

① Load - 21 mL of sample - .75 mL/min - collect FT - 7.5

② Wash - 2 V_t of Buffer 1 - collect 2 mL fractions 1 mL/min

③ & Gradient - Buffer 1 to Buffer 2 - 25 mM Tris pH 7.5
8% glycerol
5 mM Bme
.1 mM PMSF
2 M KCl

10 V_t - 400 mL gradient - linear - 1 mL/min - collect 7.5 mL fractions -

④ Wash w/ 2 V_t Buffer 2 1 mL/min - 7.5 mL fractions -

Let Column Run O/N -

Note Next time gradient should be much shallower - 1 M KCl -

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Witnessed & Understood by me,	Date	Invented by	Dat
Mary Longo	4/5/95	Recorded by	03/24/95